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CHARACTERIZATION OF HISTAMINE TYPE 1 RECEPTORS ON NATURAL SUPPRESSOR LYMPHOID CELLS*

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Abstract—Using the radioligand H_1 antagonist [^{3}H]pyrilamine, we have characterized the histamine type 1 receptor on cloned murine natural suppressor cells (NS). A single, specific binding site for [^{3}H]pyrilamine exists on these cells. The binding was saturable and reversible by various specific H_1 receptor antagonists. The rank order of potency for displacement of [^{3}H]pyrilamine binding from the H_1 receptor by H_1 receptor antagonists was promethazine = pyrobutamine > pyrilamine > diphenhydramine > chlorpheniramine. The histamine type-2 agonists, impromidine and dimaprit, and antagonists, cimetidine and ranitidine, as well as selected non-histamine agonists, did not displace [^{3}H]pyrilamine from its binding sites on the natural suppressor cells. The data indicate that the theoretical K_D was $1.1 \pm 0.3 \times 10^{-7}$ M while the measured K_D of binding for [^{3}H]pyrilamine was $6.0 \pm 0.8 \times 10^{-8}$ M; the maximum binding was 4.13 nM and the number of binding sites/cell was $2.14 \pm 0.29 \times 10^6$ (N = 3).

Histamine is a modulator of both the *in vivo* and *in vitro* models of the immune response via its direct effects on lymphoid cells [1, 2]. Histamine, like most other autacoids, affects lymphoid cells via receptors on their surface. So far, two types of cellular receptors termed H_1 and H_2 have been found to mediate the actions of histamine. Frequently H_2 receptor stimulation generates cAMP which is responsible for a number of intracellular functions [3]. While some of the H_2 receptor mediated effects on the immune system have been investigated, we do not have much information regarding the function of H_1 receptors on lymphoid cells. It has been reported that stimulation of H_1 receptors enhances contrasuppression [4].

Strober [5] has reported the appearance of naturally occurring suppressor cells in the spleens of neonatal and irradiated mice. These cells are positive for Thy 1.2, Ly-5 (CLA T200) and asialo-GM1, and negative for Lyt-1, Lyt-2, Ig, Ia and MAC-1 antigenic markers. The natural suppressor cells are present before antigenic challenge and lack antigen specificity. They may play a key role in the induction of immune tolerance. These cells have the unique ability to inhibit the antigen-specific cytolytic arms of the alloreactive immune response but leave the antigen specific suppressive arm intact. It appears that natural suppressor cells may play an important role in preventing the development of host vs graft and graft vs host diseases in allogeneic bone marrow chimeras, and in immune tolerance in the neonate and TLI (total lymphoid irradiated)-treated mice. We have shown that histamine enhanced the suppressive capacity of natural suppressor cells and that the enhancement was blocked by H_1 receptor antagonists and not by H_2 receptor antagonists [6].

In this report, we characterize histamine type-1 receptors on natural suppressor cells. We report that the H_1 receptors were very dense on these cells and had lower affinities for H_1 receptor antagonists than the same receptors in other tissues.

MATERIALS AND METHODS

Pharmacologic agents. The [^{3}H]pyrilamine used for these studies had a specific activity of 26 Ci/mmol and was purchased from the Amersham Corp. (Arlington Heights, IL). Other compounds used in this study were unlabeled pyrilamine, histamine, cimetidine, norepinephrine, epinephrine, and dopamine. All were obtained from the Sigma Chemical Co., St. Louis, MO. Impromidine, dimaprit, 2-(2-aminoethyl) thiazole dihydrochloride, and 2-pyridylethylamine were all gifts from Smith Kline & French, Herts, England. Promethazine was a gift from Wyeth Laboratories, Philadelphia, PA, and pyrobutamine was a gift from Eli Lilly & Co. Diphenhydramine and chlorpheniramine were purchased from Elkins-Sinn, Inc. (Cherry Hill, NJ) and the Spectrum Chemical Manufacturing Corp. (Gardena, CA) respectively. Prazosin HCl was purchased from Pfizer, Inc. (Groton, CT). Ranitidine was a gift from Glaxo Inc. (Research Triangle Park, NC).

Preparation of concanavalin A (Con A) supernatant (CAS) from rat spleen cells. Preparation of CAS has been described in detail [6]. Briefly, 2×10^6/ml of Lewis rat spleen cells were cultured in RPMI 1640 supplemented with 10% fetal calf serum (FCS) and 2.5 μg/ml of Con A (Pharmacia Fine Chemicals, Piscataway, NJ) for 48 hr. The supernatants were obtained and Con A was removed by using Sephadex G-25 absorption (Pharmacia Fine

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Chemicals). The supernatants were sterilized by filtration and stored at −20° until used.

Natural suppressor cells (NS). A BALB/c cloned NS cell line was a gift from Samuel Strober, M.D. (Department of Medicine, Stanford University, Stanford, CA), and was obtained as previously described [7]. Briefly, long term cultured suppressor cells were obtained from BALB/c mice that were treated with total lymphoid irradiation. One to three days after such radiation, spleen cell suspensions were made and cultured in RPMI 1640 medium with 10% FCS and 10% CAS. Later cloning was accomplished by limiting dilution [7]. The cells were maintained in long term culture on a 7 days a week feeding schedule.

S-49 lymphoma cells. S-49 lymphoma cells were maintained in continuous culture as reported previously [8].

Measurement of [^{3}H]pyrilamine binding. Binding experiments were performed by incubating 2×10^5 NS cells with different concentrations of [^{3}H]pyrilamine in a total volume of 200 μl of incubation buffer (25 mM Tris and 0.9% NaCl, pH 7.4) for various incubation periods (15–45 min) at various temperatures (4–25°). In the competition experiments, various concentrations of agonists or antagonists were added to the incubation as indicated. Incubations were terminated by rapidly diluting them with 4 ml incubation buffer at room temperature followed by vacuum filtration of the diluted incubate through Gelman Type A/E glass fiber filters. The filters were washed with 24 ml of the incubation buffer at room temperature. After drying, the filters were placed in vials, and 10 ml Scintilene (Fisher Scientific Co., Fair Lawn, NJ) was added. The filters were counted in a liquid scintillation counter (LKB Wallace, Turku, Finland). In each experiment, the nonspecific binding to cells was determined by measuring the residual amount bound when the cells were incubated in the presence of 10^{-5} M promethazine.

Data analysis. All analyses were carried out on a PROPHET 71 computer system. The saturation radioligand binding curves were analyzed by Scatchard analysis in which the regression lines were drawn by the least squares method to determine the K_D of the radioligand and the B_{max}. K_i values for the competitive unlabeled antagonist were calculated by the method of Cheng and Prusoff [9] using the equation:

$$K_i = IC_{50}/1 + (L)/K_D$$

where IC_{50} is the concentration of unlabeled antagonist which inhibits radioligand binding by 50%, (L) is the concentration of radioligand present in the assay and K_D is the equilibrium dissociation constant determined from saturation experiments. The NEWSFITSITES2 program (by Harold Perry, bolt Beranek & Newman Inc., Cambridge, MA) was used to determine the IC_{50} of the agonists and antagonists, and whether more than one class of specific binding sites was present.

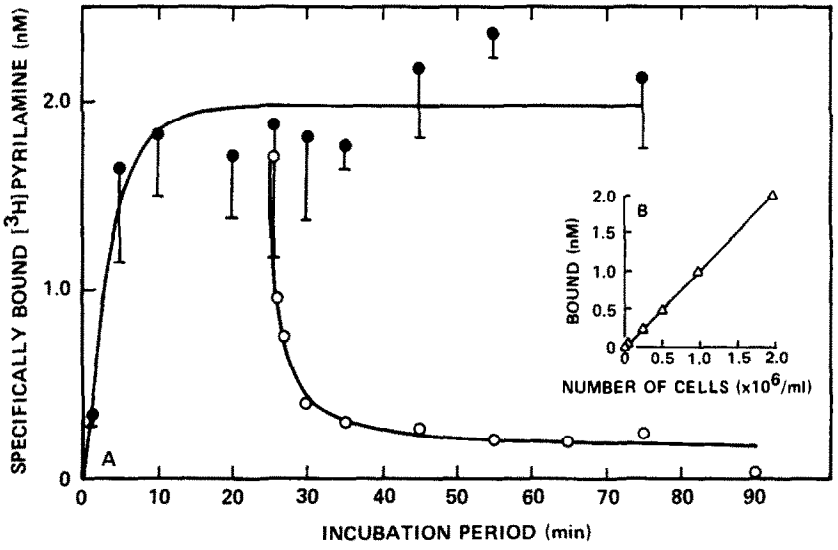

Fig. 1. (A) Time course of binding of [^{3}H]pyrilamine (●) and reversal of binding of [^{3}H]pyrilamine by 6×10^{-6} M pyrobutamine (○). In this experiment, 2×10^5 cells were incubated with [^{3}H]pyrilamine (60 nM) in the absence and presence of 10^{-5} M promethazine. Specific binding, i.e. the difference between counts bound in the absence and presence of 10^{-5} M promethazine, was determined in triplicate for each point as shown in the figure. In this experiment, 6.9% of the total binding was nonspecific. The second rate constant, K_1, was calculated from the equation $(K_{ob} - K_2)/$[^{3}H]pyrilamine, where K_2 is the rate constant for the reversal of binding (dissociation constant). For the reversal of binding (○), 2×10^5 cells were incubated with [^{3}H]pyrilamine for 25 min. Pyrobutamine was added to a final concentration of 6×10^{-6} M, and specifically bound counts were determined in triplicate at the given time. (Significance of the slope of association line $P < 0.01$, dissociation line $P < 0.05$). A representative experiment out of two is shown. (B) [^{3}H]Pyrilamine binding to increasing number of natural suppressor cells. The cell number varied, but other components were the same as those described for the association kinetics. The determinations were done in duplicate.

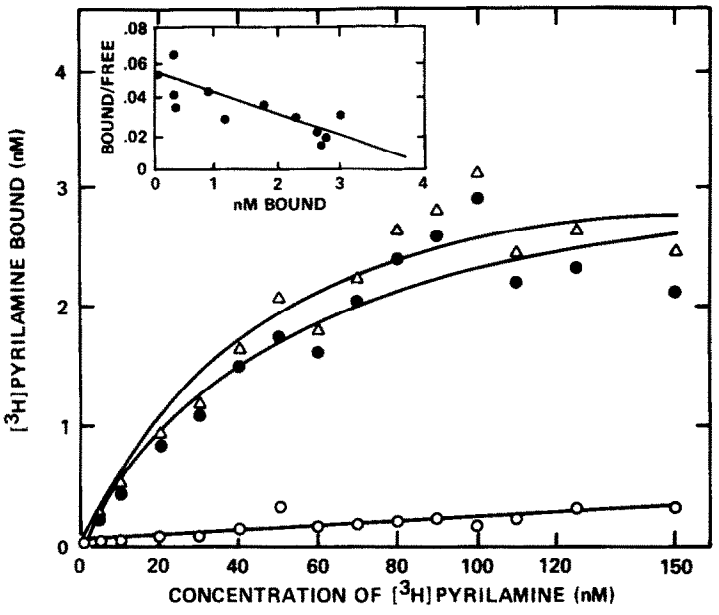

Fig. 2. Specific [^{3}H]pyrilamine binding (●) to natural suppressor cells as a function of the drug concentration. The cells (2×10^5) were incubated with increasing concentrations of [^{3}H]pyrilamine in Tris and NaCl buffer (pH 7.4) at 25° for 35 min, and specific binding was plotted at each concentration by subtracting nonspecific binding (○) from total binding (△). The figure depicts one representative experiment of three. Each point is the average of triplicate. The insert shows a Scatchard plot of [^{3}H]pyrilamine binding. The K_D of binding was 6×10^{-8} M, the maximum binding 4.13 nM, and the number of binding sites/cell 2.14×10^6 (N = 3).

RESULTS

Binding characteristics of [^{3}H]*pyrilamine to intact natural suppressor cells.* In these assays, natural suppressor cells were incubated for 30–45 min at 25° in the presence of increasing concentrations of [^{3}H]pyrilamine. We chose promethazine as an H_1 receptor antagonist used at a concentration of 10^{-5} M to estimate the extent of nonspecific binding of [^{3}H]pyrilamine. Specific binding was expressed as the difference between counts bound in the absence and presence of 10^{-5} M promethazine. Specific binding was saturable and achieved equilibrium over a 15–25 min period (Fig. 1A). This time course yielded a rate of association (K_1) of $1.64 \pm 0.36 \times 10^6\ M^{-1}\ min^{-1}$ (N = 2). Reversal of [^{3}H]pyrilamine binding by the addition of 10^{-6} M pyrobutamine indicated that specific binding reversed with a dissociation constant (K_2) of $0.17 \pm 0.01\ min^{-1}$ (N = 2) (Fig. 1A) which gave a theoretical K_D of $1.1 \pm 0.3 \times 10^{-7}$ M. The binding was linear with the increasing cell concentration (Fig. 1B). The K_D of binding was $6.0 \pm 0.8 \times 10^{-8}$ M, the maximum binding was 4.13 nM, and the number of binding sites/cell was $2.14 \pm 0.29 \times 10^6$ (N = 3) (Fig. 2). NEWFITSITES2 program analysis indicated a single class of binding sites.

Displacement of [^{3}H]*pyrilamine binding by H_1 receptor antagonists.* For the drug competition assays, the cells were incubated for 20 min with a fixed concentration of pyrilamine (30–50 nM). Five H_1 receptor antagonists, promethazine, pyrobutamine, pyrilamine, diphenhydramine and chlorpheniramine, competed for binding sites on the natural suppressor cells (Table 1). The K_i values for the respective antagonists were $5.1 \pm 1.2 \times 10^{-7}$ M (N = 5), $5.1 \pm 1.3 \times 10^{-7}$ M (N = 4), $2.5 \pm 1.0 \times 10^{-6}$ M (N = 6), $3.4 \pm 1.5 \times 10^{-6}$ M (N = 2), and $4.2 \pm 1.5 \times 10^{-6}$ M (N = 6). The rank order of potency for displacement of [^{3}H]pyrilamine binding from the H_1 receptors by various H_1 receptor antagonists was promethazine = pyrobutamine > pyrilamine > diphenhydramine > chlorpheniramine (Fig. 3).

Displacement of [^{3}H]*pyrilamine binding by H_2 receptor antagonists.* Two H_2 receptor antagonists, cimetidine and ranitidine, were employed in these studies, and they did not displace [^{3}H]pyrilamine from H_1 binding sites at concentrations as high as 10^{-4} M (Table 1).

Specificity of H_1 and H_2 agonists. Histamine H_1 agonists, 2-thiazole ethylamine and 2-pyridylethylamine, and H_2 agonists, impromidine and dimaprit, were used in these experiments. Both H_1 agonists were less potent than histamine, and no displacement of [^{3}H]pyrilamine binding to H_1 receptors of natural suppressor cells was observed when the H_2 agonists dimaprit and impromidine were used at concentrations as high as 10^{-4} M and 10^{-5} M respectively (Table 1).

Agonists unrelated to histamine, norepinephrine, epinephrine, prazosin and dopamine, did not displace [^{3}H]pyrilamine from its H_1 binding site on natural suppressor cells (Table 1). S-49 lymphoma cells were used as a negative control. No specific binding of [^{3}H]pyrilamine was observed.

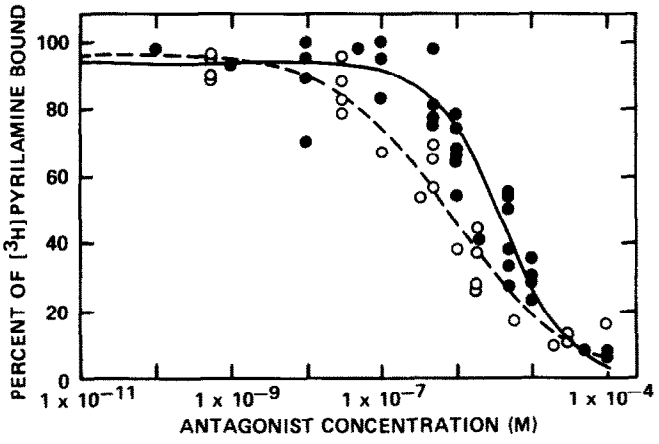

Fig. 3. Competition for [^{3}H]pyrilamine binding sites by the H_1 receptor antagonists, pyrilamine (●) and pyrobutamine (○). Various concentrations of antagonists were incubated with cells in the presence of [^{3}H]pyrilamine for 20 min. The data shown are a composite of five experiments done with pyrobutamine and six experiments done with pyrilamine. Each point is the average of triplicate determinations. The coefficient of variability of triplicate determinations was <10%.

DISCUSSION

This study confirms the presence of histamine type-1 receptors on natural suppressor cells. The receptors were very densely distributed (>2 × 10^6/cell) on the natural suppressor cells, but possessed a lower affinity for H_1 antagonists (~60 nM) than the H_1 receptors in other tissues. Chang *et al.* [10] have reported K_D values of 1.4, 1.6, 0.53, 4.0 and 0.9 nM for H_1 receptors in guinea pig illeum, adrenal gland, heart, aorta and lung respectively. The activation of H_1 receptors by histamine and other H_1 receptor agonists enhanced their suppressive capacity in MLR (mixed leukocyte reaction) [6], indicating that these receptors help to mediate a functional signal. The presence of a large number of histamine type-1 receptors on NS cells and enhanced immunosuppressive effects caused by histamine stimulation of the cells suggest that H_1 receptor mediated processes play a role in the function of these cells.

Histamine type-1 receptors have been reported previously on heterogenous mixtures of human mononuclear cells [11] and on OKT4$^+$ (Leu-3$^+$) and OKT8$^+$ (Leu-2$^+$) cells [12]. The OKT4$^+$ and T8$^+$ cells were negatively selected using solid phase immunoadsorption (panning). OKT4 and OKT8 are the T cell surface antigen markers that are present on the helper and suppressor/cytotoxic cells respectively. A disadvantage of negatively selected OKT4$^+$ and OKT8$^+$ cells is that cytotoxic T cells (OKT8$^+$, 9.3$^+$), large granular lymphocytes (Leu-15$^+$), and natural killer cells (Leu-11$^+$) are included among these negatively selected suppressor cells, which makes study of the characteristics of H_1 receptors on specific subsets of helper and suppressor T cells impossible. The clones of NS cells were homogenous,

Table 1. Inhibition of [^{3}H]pyrilamine binding by various histamine receptor related and unrelated agents

Drugs	No. of experiments	IC_{50} (μM)	K_i (M)
Promethazine	5	0.79 ± 0.18	$5.1 \pm 1.2 \times 10^{-7}$
Pyrobutamine	4	0.79 ± 0.2	$5.1 \pm 1.3 \times 10^{-7}$
Pyrilamine	6	3.88 ± 1.62	$2.5 \pm 1.0 \times 10^{-6}$
Diphenhydramine	2	5.26 ± 2.28	$3.4 \pm 1.5 \times 10^{-6}$
Chlorpheniramine	6	6.5 ± 2.4	$4.2 \pm 1.5 \times 10^{-6}$
Histamine	2	2680 ± 1020	$1.8 \times 10^{-3} \pm 6.6 \times 10^{-4}$
2-Thiazole ethylamine	5	<50% Displacement	
2-Pyridylethylamine	2	<50% Displacement	
Impromidine	3	No displacement	
Dimaprit	2	No displacement	
Cimetidine	3	No displacement	
Ranitidine	1	No displacement	
Norepinephrine	2	No displacement	
Epinephrine	2	No displacement	
Prazosin	2	No displacement	
Dopamine	2	No displacement	

Values are means ± SD.

thus the character of the H_1 receptors could be reliably studied.

While in this report we demonstrate a single specific binding site for [^{3}H]pyrilamine on a homogenous clone of lymphoid cells, Casale *et al.* [11] have reported two distinct H_1 binding sites on the mixture of human mononuclear cells. Using [^{3}H]pyrilamine binding, they have reported K_D values of 4 nM and 55 μM, corresponding to 10^4 high affinity and 10^7 low affinity receptors/mononuclear cell.

Previous to their report, Osband *et al.* [13] reported histamine binding to formaldehyde fixed lymphocytes and demonstrated the presence of a histamine receptor with a dissociation constant in the micromolar range and approximately 70,000 H_1 receptors per cell. Osband *et al.* demonstrated H_1 receptors at 37°, whereas Cameron *et al.* [12] had to use a temperature of 4° to obtain [^{3}H]pyrilamine binding on OKT4$^+$ and OKT8$^+$ cells. In their experiments, such binding was not demonstrated at 37°. In addition, Cameron *et al.* [12] described OKT8$^+$ cells as suppressor T cells, yet OKT8$^+$ cells include both the precursors of suppressor and cytotoxic T cells. Cameron *et al.* have reported K_D values of 5.0 and 44.6 nM and 6838 ± 8167 and 35,697 ± 15,468 binding sites on OKT4$^+$ and OKT8$^+$ cells respectively.

A major disadvantage of the approach of Casale *et al.* [11] is that the characteristics of H_1 receptors on the mixture of peripheral blood lymphocytes may not be representative of the subset of T cells highly enriched in the histamine type-1 receptors. In addition, each of the T cells may not possess the two distinct H_1 binding sites. A heterogenous distribution of H_1 receptors on different subsets could also explain the discrepancy in the results of Casale *et al.* versus Cameron *et al.* We have demonstrated previously that β adrenergic receptors and histamine type-2 receptors are not uniformly distributed on all the subsets of human and cloned murine T cells [2, 6, 14]. Cameron *et al.* [12] have reported a distinct distribution of histamine type-1 receptors on phenotypically and functionally distinct subsets of human lymphocytes. Their observations have confirmed our original finding that the autacoid receptors were heterogenously distributed on different lymphocyte subsets [2, 14]. A number of recent reports describe the emerging immune modulatory role of histamine type-1 receptors on lymphocytes. The H_1 receptor mediated actions include increased histidine uptake [15], increased chemotactic response of eosinophils to C5a [16], and increased expression of complement receptors on eosinophils [17]. One of the biochemical consequences of H_1 receptor ligand interaction on natural suppressor cells includes the release of a lymphokine(s). The biochemical and immunochemical characterization and the mechanism of release of this H_1 receptor mediated product(s) are under study (Khan *et al.*, manuscript in preparation).

While the stimulation of H_2 receptors on natural suppressor cells enhanced the intracellular accumulation of cAMP, such accumulation did not correlate with altered immune suppressor action. On the other hand, the transducer associated with H_1 receptors in the natural suppressor cells has not yet been established. Yet such stimulation results in enhanced immune suppression. Our preliminary data have indicated that guanylate cyclase and cGMP are not likely to be involved in the mediation of H_1 receptor dependent processes in the NS cell (unpublished observations).

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REFERENCES

1. M. M. Khan and K. L. Melmon, *Clin. Immun. Rev.* **4**, 1 (1985).
2. M. M. Khan, P. Sansoni, E. G. Engleman and K. L. Melmon, *J. clin. Invest.* **75**, 1578 (1985).
3. R. G. Coffey and J. W. Hadden, *Fedn Proc.* **44**, 112 (1985).
4. R. K. Gershon, D. D. Eardley, S. Durum, D. R. Green, F. W. Shen, K. Yamauchi, H. Cantor and D. B. Murphy, *J. exp. Med.* **153**, 1533 (1981).
5. S. Strober, *A. Rev. Immun.* **2**, 219 (1984).
6. M. M. Khan and K. L. Melmon, C. G. Fathman, B. Hertel-Wulff and S. Strober. *J. Immun.* **134**, 4100 (1985).
7. B. Hertel-Wulff, S. Okada, A. Oseroff and S. Strober, *J. Immun.* **133**, 279 (1984).
8. R. P. Rosenkranz, B. B. Hoffman, K. A. Jacobson, M. S. Verlander, L. Klevans, M. O'Donnell, M. Goodman and K. L. Melmon, *Molec. Pharmac.* **24**, 429 (1983).
9. Y. C. Cheng and W. H. Prusoff, *Biochem. Pharmac.* **22**, 3099 (1973).
10. R. S. L. Chang, V. T. Tran and S. H. Snyder, *J. Pharmac. exp. Ther.* **209**, 437 (1979).
11. T. B. Casale, S. Wescott, D. Rodbard and M. Kaliner, *Int. J. Immunopharmac.* **7**, 639 (1985).
12. W. Cameron, K. Doyle and R. E. Rocklin, *J. Immun.* **136**, 2116 (1986).
13. M. E. Osband, E. B. Cohen, B. R. Miller, Y. J. Shen, L. Cohen, L. Flescher, A. E. Brown and R. P. McCaffrey, *Blood* **58**, 87 (1981).
14. M. M. Khan, P. Sansoni, E. D. Silverman, E. G. Engleman and K. L. Melmon, *Biochem. Pharmac.* **35**, 1137 (1986).
15. J. Stewart, D. G. Jones and A. B. Kay, *Expl Immun.* **40**, 423 (1980).
16. R. A. F. Clark, J. I. Gallin and A. P. Kaplan, *J. exp. Med.* **142**, 1462 (1975).
17. A. R. E. Anwar and A. B. Kay, *J. Immun.* **121**, 1245 (1978).